

## Review Memorandum

**Reference number: 98-1396**

**Reviewed by: Mary Alice Woody, Ph. D.**

**Date:** 3/13/00

**Product:** Botulinum toxin type B (BotB) for parenteral use

**Sponsor:** Elan Pharmaceuticals

**Subject:** Licensing of manufacturing procedure for botulinum toxin type B (BotB)

## Chemistry, Manufacturing, and Controls Section Review

## 1. Introduction

**1.1. Description of the drug substance and rationale for development.** PLA 98-1396 has been submitted by Elan Pharmaceuticals for licensing of the commercial production of botulinum toxin type B (BotB) at the San Francisco, CA, facility. Other names of this drug include CAS#93384-44-2 and AN072. There are no nationally approved or pharmacopoeial names. Elan Pharmaceuticals has proposed NeuroBloc as the proprietary name for BotB, \_\_\_\_\_ . Botulinum toxin type B is intended for parenteral use to induce temporary therapeutic paralysis in muscles.

Botulinum toxin type B is produced during anaerobic fermentation of *Clostridium botulinum* serotype B. The protein is synthesized as a single polypeptide of \_\_\_\_\_ and “nicked” by proteases to form the heavy and light chains of \_\_\_\_\_ and \_\_\_\_\_ respectively, which are held together by a disulfide bond. Activation accompanies nicking, and extent of nicking correlates with potency. The toxin is associated with several nontoxic proteins that range from \_\_\_\_\_ to \_\_\_\_\_. Together with the toxin, the proteins form a complex of about \_\_\_\_\_.

The botulinum neurotoxins are zinc metalloproteases that bind receptors on neurons, and are internalized via endocytotic vesicles and released to the neuronal cytosol. The toxins cleave proteins, associated with presynaptic docking of exocytotic vesicles containing neurotransmitters, inhibiting the neurotransmitter release. Botulinum toxin type B specifically cleaves \_\_\_\_\_, and \_\_\_\_\_ at the neuromuscular junction is, resulting in paralysis that gradually reverses.

Several antigenically distinct serotypes of the botulinum neurotoxins exist, among which there is no cross-reactivity of antisera. Botulinum toxin type A is currently used to induce therapeutic muscle paralysis. Some patients that receive multiple treatments with botulinum toxin type A, however, experience reduced efficacy of the drug believed to be related to development of antibodies to the toxin. Antibodies that neutralize type A neurotoxin do not cross-react with BotB. Hence, BotB is potentially an alternative to

botulinum toxin type A for inhibition of neurotransmitter release and induction of paralysis.

Attributes of the BotB of central concern during drug manufacture are specific activity, in terms of \_\_\_\_\_ (U/ml) per concentration of toxin (ng/ml); extent of nicking, evaluated by \_\_\_\_\_ complex integrity or consistency, assessed by \_\_\_\_\_ and size exclusion chromatography; and identity, as established with neutralizing antibodies obtained from the World Health Organization.

**1.2. Summary of manufacture of botulinum toxin Type B and evaluation of raw materials.** Manufacture of BotB concentrated product (CP) at the San Francisco, CA, facility proceeds through three major steps: fermentation, recovery, and purification (Figure 1). Master batch production records for the entire manufacturing process have been included for review, and these were used for the \_\_\_\_\_ and Consistency lots.

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**1.2.2. Master Cell and Working Cell Banks.** The source of the Master Cell Bank (MCB) is \_\_\_\_\_. The seed culture history is presented in detail within this PLA submission. Briefly, *Clostridium botulinum* Type B Bean strain was isolated in 1957 at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD. This isolate had undergone several subcultures before a lyophile (\_\_\_\_\_ was acquired by \_\_\_\_\_ in 1971. The culture established at \_\_\_\_\_ was further subcultured to produce a lyophilized working (\_\_\_\_\_ ) and production seed (\_\_\_\_\_. The MCB of Elan Pharmaceuticals is derived from the production seed (\_\_\_\_\_) manufactured in August 1988 and is the source of the Working Cell Banks (WCB). The 1988 MCB at \_\_\_\_\_ was manufactured for pentavalent botulinum toxin vaccine production.

This MCB has been used to produce WCB for the Phase III clinical test materials, process validation lots, and for future commercial production. The capacity of the current MCB for commercial production is estimated to be \_\_\_\_\_. A batch production record (BPR) for production of new WCB and plans for future MCB production in 1999-2000 are included for review. The new MCB will be produced from \_\_\_\_\_.

Cell banking and fermentation media contain animal-derived components, which are of concern as potential sources of bovine spongiform encephalopathy (BSE). Media, are obtained through suppliers from \_\_\_\_\_. The animal-derived media components are ultimately from \_\_\_\_\_ licensed facilities. There are no animal materials from countries listed by the \_\_\_\_\_ as having BSE. The cooked meat medium includes \_\_\_\_\_ and \_\_\_\_\_ both of which are obtained from \_\_\_\_\_.

to reduce the likelihood of contamination by BSE. \_\_\_\_\_ medium is derived from

\_\_\_\_\_ A study that was performed to assess challenges to aseptic processing of the media, aseptic collection of in-process samples, and support of anaerobic growth was satisfactorily concluded.

The Elan Pharmaceuticals MCB was characterized by biochemical profile and Gram stain. The biochemical tests for the characterization of *C. botulinum* Type B seed culture established August 1988 \_\_\_\_\_ at \_\_\_\_\_ are not specified. However, culture purity tests, morphological tests, Gram stain procedures, and \_\_\_\_\_ automated bacterial identification tests have been specified for culture maintenance at the Elan manufacturing facility.

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**1.3.2. Shipping Procedure.** The diluted bulk is shipped to the contract filler in the same — carboy in which it was prepared. The container is packed within a sealed drum with bubble wrap and foam for protection and a temperature recorder to ensure that — is maintained. The material is shipped to the contract filler in a dedicated refrigerated truck.

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The final drug product contains  $5000 \pm 1000$  U/mL of BotB, the active ingredient. The drug product is additionally formulated with the following excipients: human serum albumin (HSA), sodium succinate, sodium chloride, \_\_\_\_\_, and \_\_\_\_\_. Monographs are provided for all chemicals, which are USP or EP grades, except for the sodium succinate (grade not specified). The biological excipient, human serum albumin (HSA), was purchased from: \_\_\_\_\_. Certificates of analysis and compliance with Agency regulations for lot \_\_\_\_\_ are included. This HSA lot has no reactivity with HBsAg, HIV-1, HIV-2, and HCV. The three vial presentations have nominal fill volumes of 0.5, 1, and 2 ml, that provide 2500, 5000, and 10,000 U, respectively.

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## **2. Process Development and Validation.**

### **2.1. Process performance validation.**

was undertaken at \_\_\_\_\_ to assess the reliability of the BotB manufacturing methods described in the Master Batch Records (MBR) and identify critical steps in the manufacturing process. In four separate fermentation and purification studies, various manufacturing processes were altered from the MBR to assess the effects on quality of the

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Fourteen protocol deviations are listed and discussed with regard to impact on the —. These appear to have had little influence on the final results or to have not adversely affected the rationale for the —. An additional Out of Specification (OOS) investigation is noteworthy. The — ratio of the — of the extreme fermentation/normal recovery and purification was above the specifications. The material was further processed before loading of the — column. Additional processing and the subsequent — column step corrected the OOS result, which suggested that the — column step is one of the critical processes in the manufacture of BotB.

**2.2. Changes in manufacturing processes from Phase III to commercial production.** Several significant changes occurred in the development of the commercial product.

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**2.3. Consistency Lots.** Three lots of BotB were manufactured at the San Francisco facility according to the Master Batch Records (MBR) to assess reproducibility of the manufacturing process. The production records for consistency lots — and — present the actual in-process methods and deviations from the MBR. One significant change in the production of lot — was the —.

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An Out of Specification (OOS) result that repeatedly occurred in the manufacture of the consistency lots was the \_\_\_\_\_, ratio that was higher than the specification. Investigation indicated that the material that increased absorbance at 260 nm was \_\_\_\_\_, and the OOS result was ascribed to variable removal of \_\_\_\_\_ during a recovery step. The inconsistent \_\_\_\_\_ content of the preparations was ultimately attributed to variations during the fermentation. The diafiltration step was extended to reduce the ratio. The downstream \_\_\_\_\_ column appears to adequately remove \_\_\_\_\_. The sponsor recommended in the submission that the \_\_\_\_\_ ratio be used for information only.

**2.3.1 Process Validation of Dilute Bulk Solution and Final Fill.** CP lot \_\_\_\_\_ was used for production of dilute bulk lot \_\_\_\_\_. Dilute bulk lots \_\_\_\_\_ were derived from CP lot \_\_\_\_\_. CP lot \_\_\_\_\_ was not processed further for license application. Dilute bulk lot \_\_\_\_\_ was used to produce final container lots C95007, C95008, and C95009. Final container lots C95010, C95011, and C95012 were derived from dilute bulk lot \_\_\_\_\_. Final container lots C95013, C95014, and C95015 were derived from dilute bulk lot \_\_\_\_\_.

Process validation for controlled and reproducible production of the dilute bulk solution, performed by Elan Pharmaceuticals, focused on shipping (discussed below) and filter validation/recovery. These are well chosen as important, perhaps the critical steps, of dilute bulk solution preparation and shipment. \_\_\_\_\_

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2.4. **Shipping validation.** A validation study was conducted by Elan to demonstrate that shipment of the dilute bulk solution to the filling facility does not compromise product quality. This study used dilute bulk solutions from the consistency lots and an earlier clinical CP. \_\_\_\_\_ was identified as the most critical factor in preservation of product quality. Maintenance of the refrigerated temperature of the cargo area of the dedicated truck and within the shipping drum was monitored during shipment. An illustration of the packing of the \_\_\_\_\_ carboy within the stainless steel shipping drum is provided within the submission. The carboy contained \_\_\_\_\_ of dilute bulk solution. Absorbent packs and temperature monitors are included in the drum, and the carboy is cushioned against shock with bubble wrap and rubber foam, as possibly excess vibration or shock could cause deterioration in product quality. Shock events were recorded during shipment. Pre- and post-shipment quality control was similar to analytical tests for the dilute bulk solution (listed below under tests for the dilute bulk solution), with the addition of an identity test and elimination of the protein concentration determination.

Results indicated that the temperature within the shipping drum could be maintained for the duration of the shipment. The temperature within the drum was — for the four shipments, and the temperature of the cargo area of the truck could be maintained within the specified —. The quality of the diluted bulk, as assessed by the analytical tests, was preserved during shipment. No pattern of change of potency was apparent in the data. Appearance, pH, and endotoxin levels were not affected. Microbial contamination of the product during shipment did not occur. Shock and vibration data for each shipment were provided, but the significance of these data is unclear because no correlation of shock with product damage is provided or perhaps is known. Minor deviations from the test protocol were discussed, and process changes were recommended. The most important of these changes was the inclusion of a plastic liner in the drum to maintain outer surface cleanliness of the carboy.

### 3. Product Testing.

Responsibilities for product testing have been distributed among Elan, — and several other laboratories. Qualification of some of the contract laboratories to perform the indicated tests is not provided.

1. Elan (San Francisco, CA) Inactive components; container closure; dilute bulk; final container biochemical and microbiological tests (LAL test)
2. Container closure; pre-filling bioburden of dilute bulk; final container microbiological tests
3. Inactive components; final container biochemical and microbiological tests (stability tests)
4. Inactive components
5. Inactive components
6. Dilute bulk (potency test); final container biochemical tests

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**3.4.3. — assay of HSA in final product.** The — assay was examined for precision, linearity, accuracy, and ruggedness. The assay was demonstrated to be — and accurate in determining concentrations of weighed amounts of HSA. Reproducibility of assay results between different analysts and different assay reagent lot numbers was demonstrated.

**3.4.4. Potency bioassay.** The point of this study was to qualify the QC group at the Eccles, San Francisco, facility to perform this assay. Toxin lots were diluted to intermediate concentrations, assayed according to SOP by several analysts, and compared against the expected values. The individual assay results were to fall within — of the target potency.

Plot of measured potency versus the expected value was — Similar results were obtained among the different analysts. These ranged from — of the expected values. The tendency of analysts at the Eccles facility to underestimate toxin potency was noted, and partly corrected by adjustment of the dilution procedures and changing to glass containers.

**3.5. Stability Testing.** The stabilities of CP, dilute bulk solution, and final container were examined under real time conditions, i.e., the recommended storage of 2-8°C, and at — which would accelerate degradation of the materials. All materials met lot release specifications at the start of these studies. Samples were analyzed periodically for appearance, pH, ( — ) potency, protein concentration, specific activity, — , percent nicking, — and bioburden.

**3.5.1. CP.** Storage of Phase III lot — manufactured at — through 12 months at — resulted in reduction of potency to — of the original and loss of — profile conformity. Potency loss was attributed to the inherent variability of the bioassay and the basis of the potency values at any time on a single assay instead of the three determinations that are routinely made. Changes in the — profile were ascribed to extractables leached from the polypropylene storage container. This is not expected to be a recurring problem because the storage containers for CP produced at the San Francisco site were extensively tested before use. The other analyses, except for the — assay (not done), passed specifications. Consistency lots — and — retained — of their original potencies after 3 months at — did not lose significant potency after 6 months at — or show other adverse changes. The potency data for — at — is more difficult to evaluate: there is a decrease in potency to — at 3 months, and the potency assay at 6 months was invalid. The three consistency lots passed all other specifications, except for the sterility test (not done except at the start). The data for — suggest a 12-month storage time at — for CP, but validation of this shelf life is pending completion of stability tests with the CP manufactured at the San Francisco site.

**3.5.2. Dilute Bulk.** Dilute bulk solutions — , and — were stored at — for 6 months without appreciable loss of potency and passed specifications for pH,

appearance, bioburden (done for all time points sampled), and residual bacterial endotoxin. A 12 month storage is supported for the dilute bulk based on the small losses of potency of Phase III lot \_\_\_\_\_

**3.5.3. Filled Containers.** Filling validation lots \_\_\_\_\_ through \_\_\_\_\_ have been held at \_\_\_\_\_ up to 3 months with essentially no adverse effects on potency. Inversion of the vials, so that the drug substance contacts the stopper, likewise had no impact on stability, as judged primarily on potency, appearance, and pH. Phase III drug lots \_\_\_\_\_ (derived from CP lot \_\_\_\_\_, \_\_\_\_\_, CP lot \_\_\_\_\_ and \_\_\_\_\_ (CP lot \_\_\_\_\_), showed little overall loss of potency after storage at \_\_\_\_\_ through 24 months, and passed the sterility and residual bacterial endotoxin tests at 18 months. Potency data varied over the 3-month testing intervals. However, substantial losses in potency were observed in the Phase III lots when these were stored for one year or less at \_\_\_\_\_. The data suggest a 24-month expiration for the drug substance in the final container when stored at \_\_\_\_\_ pending completion of stability testing of the filling validation lots.

**4. In Vivo Tests for Bioequivalency.** Nonclinical evaluations were undertaken to compare Phase III drug manufactured at the \_\_\_\_\_ facility with BotB manufactured at the San Francisco commercial production facility. Three animal models were chosen for these studies based on physiological similarity of the neuromuscular junctions to those of humans; the relevance of the in vivo assay to pharmacological responses in humans; the identical mechanism of action of the toxin as in humans; reproducibility of the tests results; and the ability to demonstrate bioequivalence of BotB from the two sources.

**4.1. Mouse chemodenervation model.** This is a hind-limb paralysis assay to assess \_\_\_\_\_

Assessment of paralysis was made independently by several observers to compensate for the inherent subjective nature of these measurements.

A preliminary study was conducted to assess the time points for scoring of paralysis and appropriate doses for the definitive study, and to refine methods to make the assay as reproducible as possible. The purpose of the definitive study was to compare BotB manufactured at the San Francisco facility with that manufactured for Phase III clinical trials. Dose response curves for each preparation of BotB were compared and found to be similar in slope and regression equation, suggesting that the toxin from the two sources were bioequivalent.

#### 4.2. Monkey comparability model.

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The following bioequivalence test was designed, based on the preliminary results, to compare BotB manufactured at the San Francisco facility with Phase III material prepared at

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Electrophysiologic measurements to assess paralysis indicated that the two BotB preparations were equivalent.

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**4.3. Monkey diffusion model.** The purpose of these studies was to determine the extent to which neighboring neuromuscular junctions are affected by injection of a muscle with BotB. This is a clinically significant point because diffusion into other sites could be a source of adverse events for patients undergoing treatment with the neurotoxin.

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#### 5. Comments and questions for the manufacturer:

1. How is — which is purchased in carboys, distributed and used to maintain its quality while formulating buffers and cleaning glassware?
  - a. — has been specified at times in PLA 98 1396 (example, p.347, volume 1, for storage and rinsing of a column). Please clarify which grade of water has been in use throughout development and manufacture of BotB.
  - b. Has the supplier of — been certified?

- c. Please clarify the differences between water for injection (WFI) and water for irrigation (WFIr).
- d. With reference to the — column packing and testing (p. 332, vol. 1) how much — was used to rinse the — column after cleaning with — and how is cleaning monitored during that process to assure that the — s adequately removed?
2. Has the human serum albumin (HSA) that has been used for formulating the final product and development of size exclusion chromatography methods for purification of BotB (p. 341, vol. 1) been certified or demonstrated to not be a potential source of blood-borne adventitious agents?
- a. Was — used for testing of the new : — column noted in the batch production record for CP lot — p. 92, volume 3)?
- b. Please clarify when use of — was stopped and — was substituted as a test of column performance.
3. What biochemical tests, referred to on page 71 (vol. 1), were performed on the *C. botulinum* cultures from which the 1988 Master Cell Banks were derived?
4. Do tests of culture purity include an identity test with specific antisera to demonstrate that the *C. botulinum* type B Bean culture produces only type B botulinum neurotoxin?
5. How was the — shelf life for the working cell bank determined?
6. Please clarify the rationale for storage of the MCB in a — whereas the WCB is stored —
7. Please clarify whether the — of the culture occurs in the fermentor or in another vessel (p. 151, vol. 2, vs. p. 62, vol. 1). If the — is performed in the stainless steel fermentor, does the fermentor have liner or inner coating that would prevent leaching of metal ions into the culture by the corrosive action of the —
8. Why was no "normal fermentation and recovery/normal purification" performed during the — and how were "normal" conditions defined?
9. Please clarify why the CP release specifications for percent nicking is greater than —, whereas the acceptance criteria is — (Table 6, p. 159, vol. 2).
10. How were the various protein assays chosen, and why are different assays used at different points in the drug manufacture?

11. Is there a test for identity of the diluted bulk solution? Were there in vitro tests of identity (i.e., Western blots or ELISA with type B-specific antisera) done on the concentrated product?
12. Total heterotrophic microbial counts, determined by a pour plate or membrane filtration method, is used as a measure of bioburden.
  - a. Please clarify whether both methods are used on the same samples, or if there are instances in which one test method is preferred over the other.
  - b. Please clarify which organisms, listed on p. 127 of volume 2, are used for the fungistasis/bacteriostasis tests by writing genus names in full.
13. Please clarify why the permissible temperature range of the shipping drum, (p. 22, vol. 5), is greater than the range permitted for the truck,
14. Please explain the increase in temperature after the end of shipment recorded for dilute bulk batch p.237, and p. 241, vol. 5. Was the product transferred to refrigerated storage before the temperature increase?
15. Because filling line no. 2, room 163, is used for other drugs than Neurobloc (p. 137, vol. 4), how is the filling line decontaminated before and after Neurobloc filling to ensure that drugs are not mixed?
16. Why are the filled vials that are yet to be inspected (p. 153, vol. 4) held at and not 2-8°C?
17. For the media fill of the final product vials, described pages 95-101 volume 5, was an anaerobic test organism used?
18. For the sterile media fill described on pages 95-101 volume 5, how was it decided to use

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